REMARKS/ARGUMENTS

The foregoing amendments in the specification and claims are of a formal nature, and do not add new matter.

Prior to the present amendment, Claims 28-47 were pending in this application and were rejected on various grounds. With this amendment, Claims 36-37 and 41-43 have been canceled without prejudice, Claims 28-35, 38-39 and 44 have been amended, and new Claims 48-52 have been added.

Claims 28-35, 38-40 and 44-52 are pending after entry of the instant amendment. Applicants expressly reserve the right to pursue any canceled matter in subsequent continuation, divisional or continuation-in-part applications.

The amendments to the specification and claims are fully supported by the specification and claims as originally filed and do not constitute new matter. In addition, new Claims 48-52 are fully supported by the specification as originally filed. Amendments to Claims 28-32 can be found in Example 145 at least on page 509, line 24 of the specification. Support new Claims 48-52 can be found at least in Example 151, starting on page 512, line 28 of the specification.

In addition, Applicants request the PTO to take note of the Revocation and Power of Attorney and Change of Address filed on February 28, 2003, and kindly direct all future correspondence to the address indicated, *i.e.*, to:

CUSTOMER NO. 35489
Ginger R. Dreger
Heller Ehrman White & McAuliffe LLP
275 Middlefield Road
Menlo Park, California 94025
Telephone: (650) 324-7000
Facsimile: (650) 324-0638

Specification

The specification has been amended to remove embedded hyperlink and/or other form of browser-executable code.

Further, Applicants have amended the specification to comply with the provisions of the Budapest Treaty.

Priority

Applicants rely on the mouse kidney mesangial cell proliferation assay (Example 145, Assay #92) and induction of pancreatic β-cell precursor proliferation assay (Example 151, Assay #117) for support of patentable utility.

The data for the mouse kidney mesangial cell proliferation assay and induction of pancreatic β-cell precursor proliferation assay were first disclosed in International Application Serial No. PCT/US00/04342, filed on February 18, 2000, the priority of which is claimed in the present application. In support, Applicants enclose herewith pages 520-521 and 523-524, describing the mouse kidney mesangial cell proliferation assay (Example 145, Assay #92) and induction of pancreatic β-cell precursor proliferation assay (Example 151, Assay #117), of PCT Publication WO 00/78961, corresponding to PCT Application PCT/US00/04342.

Claim Rejections – 35 U.S.C. §101

Claims 28-47 are rejected under 35 U.S.C. §101, allegedly because the claimed invention lacks patentable utility. Examiner notes that "there are two cited utilities in the specification .... The first utility is ... assay 92, ... a mouse kidney mesangial cell proliferation assay ... [and] the second utility is assay 117, ... induction of pancreatic β-cell precursor proliferation [assay]. These utilities are credible." However, the Examiner alleges that the cited utilities lack substantial and specific asserted utility, because "[t]here is no evidence that function in these assays is relevant to any real world use, such as disease therapy", and "[t]he protein is not shown to treat disease, to correct any condition or to provide any useful information whatsoever."

Applicants respectfully disagree and traverse the rejection.

Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot.

Utility – Legal Standard

According to the Utility Examination Guidelines ("Utility Guidelines"), 66 Fed.

Reg. 1092 (2001) an invention complies with the utility requirement of 35 U.S.C. §101, if it has at least one asserted "specific, substantial, and credible utility" or a "well-established utility."

Under the Utility Guidelines, a utility is "specific" when it is particular to the subject matter claimed. For example, it is generally not enough to state that a nucleic acid is useful as a diagnostic without also identifying the conditions that is to be diagnosed.

The requirement of "substantial utility" defines a "real world" use, and derives from the Supreme Court's holding in *Brenner v. Manson*, 383 U.S. 519, 534 (1966) stating that "[t]he basic quid pro quo contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility." In explaining the "substantial utility" standard, M.P.E.P. §2107.01 cautions, however, that Office personnel must be careful not to interpret the phrase "immediate benefit to the public" or similar formulations used in certain court decisions to mean that products or services based on the claimed invention must be "currently available" to the public in order to satisfy the utility requirement. "Rather, **any reasonable use that an applicant has identified for the invention that can be viewed as providing a public benefit should be accepted as sufficient**, at least with regard to defining a "'substantial'" utility.'" M.P.E.P. §2107.01, emphasis added. Indeed, the Guidelines for Examination of Applications for Compliance With the Utility Requirement, set forth in M.P.E.P. §2107 II(B)(1) gives the following instruction to patent examiners: "If the applicant has asserted that the claimed invention is useful for any particular practical purpose . . . and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility."

Finally, the Utility Guidelines restate the Patent Office's long established position that any asserted utility has to be "credible." "Credibility is assessed from the perspective of one of ordinary skill in the art in view of the disclosure and any other evidence of record . . . that is probative of the applicant's assertions." M.P.E.P. §2107 II(B)(1)(ii). Such a standard is presumptively satisfied unless the logic underlying the assertion is seriously flawed, or if the facts upon which the assertion is based are inconsistent with the logic underlying the assertion. Revised Interim Utility Guidelines Training Materials, 1999.

Proper Application of the Legal Standard

As discussed above, Applicants rely on the mouse kidney mesangial cell proliferation assay (Example 145, Assay #92) for support of patentable utility. The assay was first disclosed in International Application Serial No. PCT/US00/04342, filed on February 18, 2000, the priority of which is claimed in the present application.

The mouse kidney mesangial cell proliferation assay is designed to show that whether a polypeptide, such as PRO1382, is capable of inducing or inhibiting proliferation of mammalian kidney mesangial cells. Therefore, the assay identifies polypeptides that are expected to be useful for treating disorders associated with decreased mesangial cell function, such as Berger disease, or other nephropathies associated with Schonlein-Henoch purpura, celiac disease, dermatitis herpetiformis or Crohn disease.

The assay is performed as follows: mouse kidney mesangial cells were plated on a 96 well plate in growth media (3:1 mixture of DMEM and Ham's 12 medium, 95% fetal bovine serum, 5% supplemented with 14 mM HEPES) and grown overnight. On day 2, the PRO1382 polypeptide was diluted at 2 concentrations (1% and 0.1%) in serum-free medium and added to the cells. Control samples were serum free medium alone. On day 4, 20ul of the Cell Titer 96 Aqueous one solution reagent (Progema) was added to each well and the colormetric reaction was allowed to proceed for 2 hours. The absorbance was then measured at 490nm. A positive in the assay was anything that gives an absorbance reading which is at least 15% *above* the control reading. Therefore, PRO1382 was tested positive for inducing proliferation of mammalian kidney mesangial cells in this assay.

Applicants respectfully submit that mesangial cells are specialized pericytes which can secrete or respond to a wide array of growth factors, hormones, and cytokines. Applicants further submit that at the effective filing date of the present application, it was well-known in the art that mammalian kidney mesangial cells activated by inflammatory events can participate in the interactive process that lead to glomerulonephritis (Kashgarian *et al.*, *Lab. Invest.* 52: 569-571 (1985) – copy enclosed). For example, it was known in the art that proliferation of glomerular mesangial cells and their production of additional extracellular matrix are involved in the pathophysiology of glomerulosclerosis, proteinuria, and progressive renal failure (Striker *et al.*,

Semin. Nephrol. 9: 318-328 (1989) – copy enclosed).

At the effective filing date of the present application, mammalian mesangial cell proliferation assay had been widely used to detect molecules that modulate the proliferation of mesangial cells and to identify potential therapeutic agents for treating kidney disease. For instance, it was demonstrated, as early as in 1992, that various cytokines, such as EGF, PDGF, IGF, insulin, TGF-alpha, IL-1, 4, and 6, endothelin, prostaglandin F2, and angiotensin II, induce mitogenesis in cultured mesangial cells, indicating a correlation between cytokines and kidney disease (Mene *et al.*, *Physiol. Rev.* 69: 1347-1424 (1989); Ruef *et al.*, *Kidney Int.* 38: 249-257 (1990); Wolf *et al.*, *Am. J. Pathol.* 140: 95-107 (1992) – copies enclosed).

In addition, Eto *et al.* suggests that sarpogrelate, a 5-hydroxytryptamine receptor subtype 2A (5-HT_{2A}) antagonist, may be effective in the treatment of some glomerulonephriris associated with mesangial cell proliferation based on the anti-mitogenic effect of sarpogrelate in cultured rat mesangial cells. (Eto *et al.*, Life Sciences 60: 193-199 (1997) – copy enclosed).

Recent studies further confirmed the reliability of the mammalian mesangial cell proliferation assay in identifying therapeutic agents for treating kidney diseases. Gohda *et al.* found that antiplatelet agents, such as diprydamole and dilazep hydrochloride, inhibit lipopolysaccharide-induced mouse mesangial cell IL-6 secretion and proliferation. It is well known in the art that antiplatelet agents are widely used to reduce proteinuria and to prevent the progression of chronic glomerulonephritis or diabetic nephropathy to end-stage renal failure (Gohda *et al.*, *Kidney Blood Press Res.* 24:33-38 (2001) – copy enclosed). Similarly, Ono *et al.* indicated that benidipine may be effective to suppress the progression of mesangioproliferative glomerular disease because this compound markedly inhibited the proliferation of cultured human mesangial cells (Ono *et al.*, *Am J. Nephrol.* 22: 581-586 (2002) – copy enclosed). Accordingly, a variety of real-life utilities are envisioned for PRO1382 and its encoding nucleic acids based on the mouse kidney mesangial cell proliferation assay results disclosed herein.

Applicants further rely on the induction of pancreatic β-cell precursor proliferation assay (Example 151) for priority and to establish patentable utility for polypeptide PRO1382. These results were first disclosed in International Application Serial No. PCT/US00/04342, filed on February 18, 2000, priority to which has been claimed in this application.

The pancreatic β-cell precursor proliferation assay is designed to show that whether a polypeptide, such as PRO1382, is capable of inducing an increase in the number of pancreatic β-cell precursor cells. Therefore, the assay identifies polypeptides that are expected to be useful for treating disorders associated with decreased β-cell function, such as diabetes mellitus.

The assay uses a primary culture of mouse fetal pancreatic cells and an alteration in the expression of markers that represent either β-cell precursors or mature β-cells are measured. Marker expression is measured by real time quantitative PCR (RTQ-PCR); wherein the marker being evaluated is a transcription factor called Pdx1.

Here, the pancreata were dissected from E14 embryos (CD1 mice) and then digested with collagenase/dispase at 37°C for 40 to 60 minutes. The digestion was then neutralized with an equal volume of 5% BSA and the cells were washed once with RPMI1640. At day 1, the cells are seeded into 12-well tissue culture plates (pre-coated with laminin, 20μg/ml in PBS, Boehringer Mannheim, #124317). Cells from pancreata from 1-2 embryos were distributed per well. At day 2, the media was removed and the attached cells washed with RPMI/1640. Two mls of minimal media were added in addition to the PRO1382 polypeptide. At day 4, the media was removed and RNA prepared from the cells and marker expression were analyzed by real time quantitative RT-PCR. PRO1382 was considered to be active in the assay since an increase in the expression of the relevant β-cell marker, Pdx1, was observed compared to the untreated controls.

Applicants respectfully submit that at the effective filing date of the present application, it was well-known in the art that the pancreatic β-cell plays a central role in the regulation of glucose homeostasis and that insufficient insulin release in response to elevated glucose levels is a hallmark of diabetes. (Ahlgren *et al., Genes & Develop.* 12: 1763-1768 (1998) - copy enclosed). It was also well known at the effective filing date of the present application that the homeodomain transcription factor Pdx1 is required for pancreas development, including the differentiation and function of β-cells. (Offield *et al.*, *Development* 122: 983-995 (1996) - copies enclosed). Accordingly, based on the general knowledge in the art at time the invention was made, Applicants respectfully submit that Pdx1 was a well-known and widely accepted β-cell marker.

Ahlgren *et al.* found that inactivation of the homeodomain protein Pdx1 gene in mice resulted in loss of the β-cell phenotype and as a result, these mice developed diabetes. Accordingly, the authors concluded that expression of Pdx1 is critical for maintaining the hormone-producing phenotype and homeostatic regulation of the glucose-sensing system in β-cells. (Ahlgren *et al., Genes & Develop.* 12: 1763-1768 (1998)).

Recent studies further confirmed that Pdx1 deficiency results in pancreatic β-cell dysfunction and diabetes mellitus. (See *e.g.,* Boj *et al., PNAS* 98(25): 14481-14486 (2001); Leibowitz *et al., Diabetes* 50: 1799-1806 (2001)). Furthermore, Zalzman *et al.* showed that fetal human progenitor liver cells (FH) with an increase in the expression of Pdx1 gene activated multiple β-cell genes, produced and stored considerable amounts of insulin and restored euglycemia and hyperglycemic immunodeficient mice. Based on these results, the authors concluded that activating the insulin producing β-cells by expressing Pdx1 may offer a way to treat patients with type 1 diabetes. (Zalzman *et al., PNAS* 100(12): 7253-7259 (2003). Therefore, these references confirm that increasing the expression of Pdx1, a key regulator of insulin expression in β-cells, would result in activation of β-cells.

Accordingly, Applicants respectfully submit that molecules, such as PRO1382, which increase the expression of Pdx1, would also be capable of inducing proliferation of pancreatic β-cell precursor cells. Thus, a variety of real-life utilities, such as treatment of diabetes, are envisioned for PRO1382 and its encoding nucleic acids based on the pancreatic β-cell precursor proliferation assay results disclosed herein.

Accordingly, regarding Examiner's assertion that "[t]he claimed assays are not clinically relevant since there is no evidence of record that function as a mitogen in either assay will yield a protein that will function to achieve any therapeutic function", Applicants respectfully submit that, as discussed above, there is a clear nexus between the assay results and the real world uses for the PRO1382 polypeptide and its encoding nucleic acids. Further, as set forth in M.P.E.P. §2107 II(B)(1), if the applicant has asserted that the claimed invention is useful for any particular practical purpose, and the assertion would be considered credible by a person of ordinary skill in the art, a rejection based on lack of utility should not be imposed. The logic underlying the asserted utilities in the present case is not inconsistent with general knowledge in the art, and

would be considered credible by a person skilled in the art. It is, of course, always possible that an invention fails on its way of development to a commercial product. Thus, despite recent advances in rational drug design, a large percentage of drug candidates fails, and never makes it into a drug product. However, the USPTO is not the FDA, the law does not require that a product (drug or diagnostic) be currently available to the public in order to satisfy the utility requirement.

In view of the above, Applicants respectfully submit that PRO1382 and its encoding nucleic acids have several real life utilities, including use in treating diabetes, Berger disease, or other nephropathies associated with Schonlein-Henoch purpura, celiac disease, dermatitis herpetiformis or Crohn disease, and that the specification discloses at least one credible, substantial and specific utility for the polypeptide PRO1382 and its encoding nucleic acids. Accordingly, the Examiner is requested to reconsider and withdraw the present rejection under 35 U.S.C. §101.

Claim Rejections Under 35 U.S.C. §112, First Paragraph (Enablement)

Claims 28-47 were rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The rejection is based in part on the perceived unpredictability of the relevant art with regard to protein function, and partly on the assertion that "Applicants has not demonstrated a nexus between the function of the assay and the utility of the [PRO1382] protein."

Applicants disagree and respectfully traverse the rejection.

Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot.

In response to the previous "lack of utility" rejection, Applicants have shown that the claimed polypeptides and the encoding nucleic acids do have at least two patentable utility, namely utility in inducing proliferation of mammalian kidney mesangial cells and inducing proliferation of pancreatic β-cell precursor cells. All genus claims have been amended to recite

that "the encoded polypeptide induces proliferation of kidney mesangial cells" or "the encoded polypeptide induces proliferation of pancreatic β-cell precursor cells." Coupled with the general knowledge in the art at the time of the invention, Applicants submit that the present application provides sufficient guidance to one skilled in the art to use the invention without undue experimentation. As the M.P.E.P. states, "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Certain Limited-charge cell Culture Microcarriers, 221 USPQ 1165, 1174* (Int'l Trade Comm'n 1983), *aff'. sub nom., Massachusetts Institute of Technology v A.B. Fortia,* 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985) M.P.E.P. 2164.01. The Examiner is therefore, respectfully requested to reconsider and withdraw the rejection of these claims under 35 U.S.C. §112, first paragraph.

Claim Rejections - 35 U.S.C. §112 - Written Description

Claims 28-37 and 41-47 have been rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner further alleges that the claims encompass "hybridization" language without any correlative function.

Applicants respectfully disagree and traverse the rejection.

Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot.

Without acquiescing to the Examiner's position in the current rejections, and without prejudice to further prosecution of the subject-matter in one or more continuation or divisional applications, Claims 28-32 (and, as a consequence, those claims dependent from the same) now recite that the encoded polypeptide "induces proliferation of kidney mesangial cells". Thus, this biological activity, coupled with a well defined, and relatively high degree of sequence identity are believed to sufficiently define the claimed genus, such that one skilled in the art would readily recognize that the Applicants were in the possession of the invention claimed at the effective filing date of this application. Hence, the present rejection should be withdrawn.

Claim Rejections – 35 U.S.C. §102

Claims 41-43 are rejected under 35 U.S.C. §102(b) as being anticipated by Urade *et al.* (PNAS 88:1069-1073 (1991)).

Without acquiescing to the Examiner's position in the current rejections, and without prejudice to further prosecution of the subject-matter in one or more continuation or divisional applications, Applicants submit that the cancellation of Claims 41-43 renders the rejection of these claims moot. Hence, the present rejection should be withdrawn.

CONCLUSION

All claims pending in the present application are believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641**, referencing Attorney's Docket No. **39780-2830 P1C54**). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: November 8, 2004

By: _____
Anna L. Barry (Reg. No. 51,436)

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The basic protocol for this assay is described in Current Protocols in Immunology, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Insitutes of Health, Published by John Wiley & Sons, Inc.

More specifically, in one assay variant, peripheral blood mononuclear cells (PBMC) are isolated from mammalian individuals, for example a human volunteer, by leukopheresis (one donor will supply stimulator PBMCs, the other donor will supply responder PBMCs). If desired, the cells are frozen in fetal bovine serum and DMSO after isolation. Frozen cells may be thawed overnight in assay media (37°C, 5% CO_2) and then washed and resuspended to $3x10^6$ cells/ml of assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate). The stimulator PBMCs are prepared by irradiating the cells (about 3000 Rads).

The assay is prepared by plating in triplicate wells a mixture of:

100:1 of test sample diluted to 1% or to 0.1%,

50 :1 of irradiated stimulator cells, and

50 :1 of responder PBMC cells.

100 microliters of cell culture media or 100 microliter of CD4-IgG is used as the control. The wells are then incubated at 37°C, 5% CO_2 for 4 days. On day 5, each well is pulsed with tritiated thymidine (1.0 mC/well; Amersham). After 6 hours the cells are washed 3 times and then the uptake of the label is evaluated.

In another variant of this assay, PBMCs are isolated from the spleens of Balb/c mice and C57B6 mice. The cells are teased from freshly harvested spleens in assay media (RPMI; 10% fetal bovine serum, 1% penicillin/streptomycin, 1% glutamine, 1% HEPES, 1% non-essential amino acids, 1% pyruvate) and the PBMCs are isolated by overlaying these cells over Lympholyte M (Organon Teknika), centrifuging at 2000 rpm for 20 minutes, collecting and washing the mononuclear cell layer in assay media and resuspending the cells to $1x10^7$ cells/ml of assay media. The assay is then conducted as described above.

Positive increases over control are considered positive with increases of greater than or equal to 180% being preferred. However, any value greater than control indicates a stimulatory effect for the test protein.

The following PRO polypeptides tested positive in this assay: PRO1246 and PRO1343.

EXAMPLE 145: Mouse Kidney Mesangial Cell Proliferation Assay (Assay 92)

This assay shows that certain polypeptides of the invention act to induce proliferation of mammalian kidney mesangial cells and, therefore, are useful for treating kidney disorders associated with decreased mesangial cell function such as Berger disease or other nephropathies associated with Schönlein-Henoch purpura, celiac disease, dermatitis herpetiformis or Crohn disease. The assay is performed as follows. On day one, mouse kidney mesangial cells are plated on a 96 well plate in growth media (3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium, 95% fetal bovine serum, 5% supplemented with 14 mM HEPES) and grown overnight. On day 2, PRO polypeptides are diluted at 2 concentrations(1% and 0.1%) in serum-free medium and added to the cells. Control samples are serum-free medium alone. On day 4, 20μl of the Cell Titer 96 Aqueous one solution reagent (Progema) was added to each well and the colormetric reaction was allowed to proceed for 2 hours. The absorbance (OD) is then measured at 490 nm. A positive in the assay is anything that gives an absorbance reading which is at least 15% above the control reading.

The following polypeptide tested positive in this assay: PRO1265, PRO1244 and PRO1382:

EXAMPLE 146: Induction of Pancreatic β-Cell Precursor Differentiation (Assay 89)

This assay shows that certain polypeptides of the invention act to induce differentiation of pancreatic β-cell precursor cells into mature pancreatic β-cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent either β-cell precursors or mature β-cells. Marker expression is measured by real time quantitative PCR (RTQ-PCR); wherein the marker being evaluated is insulin.

The pancreata are dissected from E14 embryos (CD1 mice). The pancreata are then digested with collagenase/dispase in F12/DMEM at 37°C for 40 to 60 minutes (collagenase/dispase, 1.37 mg/ml, Boehringer Mannheim, #1097113). The digestion is then neutralized with an equal volume of 5% BSA and the cells are washed once with RPMI1640. At day 1, the cells are seeded into 12-well tissue culture plates (pre-coated with laminin, 20μg/ml in PBS, Boehringer Mannheim, #124317). Cells from pancreata from 1-2 embryos are distributed per well. The culture medium for this primary cuture is 14F/1640. At day 2, the media is removed and the attached cells washed with RPMI/1640. Two mls of minimal media are added in addition to the protein to be tested. At day 4, the media is removed and RNA prepared from the cells and marker expression analyzed by real time quantitative RT-PCR. A protein is considered to be active in the assay if it increases the expression of the relevant β-cell marker as compared to untreated controls.

14F/1640 is RPMI1640 (Gibco) plus the following:

group A 1:1000

group B 1:1000

recombinant human insulin 10 μg/ml

Aprotinin (50μg/ml) 1:2000 (Boehringer manheim #981532)

Bovine pituitary extract (BPE) 60μg/ml

Gentamycin 100 ng/ml

Group A : (in 10ml PBS)

Transferrin, 100mg (Sigma T2252)

Epidermal Growth Factor, 100μg (BRL 100004)

Triiodothyronine, 10μl of $5x10^{-6}$ M (Sigma T5516)

Ethanolamine, 100μl of 10^{-1} M (Sigma E0135)

Phosphoethalamine, 100μl of 10^{-1} M (Sigma P0503)

Selenium, 4μl of 10^{-1} M (Aesar #12574)

Group C : (in 10ml 100% ethanol)

Hydrocortisone, 2μl of $5X10^{-3}$ M (Sigma #H0135)

Progesterone, 100μl of $1X10^{-3}$ M (Sigma #P6149)

Forskolin, 500μl of 20mM (Calbiochem #344270)

Minimal media:

EXAMPLE 149: Detection of PRO Polypeptides That Affect Glucose or FFA Uptake by Primary Rat Adipocytes (Assay 94)

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by adipocyte cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by adipocytes would be beneficial including, for example, obesity, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat adipocytes, and allowed to incubate overnight. Samples are taken at 4 and 16 hours and assayed for glycerol, glucose and FFA uptake. After the 16 hour incubation, insulin is added to the media and allowed to incubate for 4 hours. At this time, a sample is taken and glycerol, glucose and FFA uptake is measured. Media containing insulin without the PRO polypeptide is used as a positive reference control. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as stimulators of glucose and/or FFA uptake in this assay: PRO1265, PRO1283, PRO1279, PRO1303, PRO1306, PRO1325, PRO1565 and PRO1567.

The following PRO polypeptides tested positive as inhibitors of glucose and/or FFA uptake in this assay: PRO1194, PRO1190, PRO1326, PRO1343, PRO1480, PRO1474, PRO1575 and PRO1760.

EXAMPLE 150: Chondrocyte Re-differentiation Assay (Assay 110)

This assay shows that certain polypeptides of the invention act to induce redifferentiation of chondrocytes, therefore, are expected to be useful for the treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis. The assay is performed as follows. Porcine chondrocytes are isolated by overnight collagenase digestion of articulary cartilage of metacarpophalangeal joints of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm^2 in Ham F-12 containing 10% FBS and 4 μg/ml gentamycin. The culture media is changed every third day and the cells are then seeded in 96 well plates at 5,000 cells/well in 100μl of the same media without serum and 100 μl of the test PRO polypeptide, 5 nM staurosporin (positive control) or medium alone (negative control) is added to give a final volume of 200 μl/well. After 5 days of incubation at 37°C, a picture of each well is taken and the differentiation state of the chondrocytes is determined. A positive result in the assay occurs when the redifferentiation of the chondrocytes is determined to be more similar to the positive control than the negative control.

The following polypeptide tested positive in this assay: PRO1265, PRO1250, PRO1430, PRO1356, PRO1275, PRO1274, PRO1286, PRO1273, PRO1283, PRO1279, PRO1306, PRO1325, PRO1343, PRO1418, PRO1565, PRO1474, PRO1787, PRO1556 and PRO1801.

EXAMPLE 151: Induction of Pancreatic β-Cell Precursor Proliferation (Assay 117)

This assay shows that certain polypeptides of the invention act to induce an increase in the number of pancreatic β-cell precursor cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent

either β-cell precursors or mature β-cells. Marker expression is measured by real time quantitative PCR (RTQ-PCR); wherein the marker being evaluated is a transcription factor called Pdx1.

The pancreata are dissected from E14 embryos (CD1 mice). The pancreata are then digested with collagenase/dispase in F12/DMEM at 37°C for 40 to 60 minutes (collagenase/dispase, 1.37 mg/ml, Boehringer Mannheim, #1097113). The digestion is then neutralized with an equal volume of 5% BSA and the cells are washed once with RPMI1640. At day 1, the cells are seeded into 12-well tissue culture plates (pre-coated with laminin, 20μg/ml in PBS, Boehringer Mannheim, #124317). Cells from pancreata from 1-2 embryos are distributed per well. The culture medium for this primary cuture is 14F/1640. At day 2, the media is removed and the attached cells washed with RPMI/1640. Two mls of minimal media are added in addition to the protein to be tested. At day 4, the media is removed and RNA prepared from the cells and marker expression analyzed by real time quantitative RT-PCR. A protein is considered to be active in the assay if it increases the expression of the relevant β-cell marker as compared to untreated controls.

14F/1640 is RPMI1640 (Gibco) plus the following:

group A 1:1000

group B 1:1000

recombinant human insulin 10 μg/ml

Aprotinin (50μg/ml) 1:2000 (Boehringer manheim #981532)

Bovine pituitary extract (BPE) 60μg/ml

Gentamycin 100 ng/ml

Group A : (in 10ml PBS)

Transferrin, 100mg (Sigma T2252)

Epidermal Growth Factor, 100μg (BRL 100004)

Triiodothyronine, 10μl of $5x10^{-6}$ M (Sigma T5516)

Ethanolamine, 100μl of 10^{-1} M (Sigma E0135)

Phosphoethalamine, 100μl of 10^{-1} M (Sigma P0503)

Selenium, 4μl of 10^{-1} M (Aesar #12574)

Group C : (in 10ml 100% ethanol)

Hydrocortisone, 2μl of $5X10^{-3}$ M (Sigma #H0135)

Progesterone, 100μl of $1X10^{-3}$ M (Sigma #P6149)

Forskolin, 500μl of 20mM (Calbiochem #344270)

Minimal media:

RPMI 1640 plus transferrin (10 μg/ml), insulin (1 μg/ml), gentamycin (100 ng/ml), aprotinin (50 μg/ml) and BPE (15 μg/ml).

Defined media:

RPMI 1640 plus transferrin (10 μg/ml), insulin (1 μg/ml), gentamycin (100 ng/ml) and aprotinin (50 μg/ml).

The following polypeptides tested positive in this assay: PRO1382 and PRO1561.